Several extraction procedures available in addition to PCA extraction. Depending on the nature of the metabolites, one of the alternatives may be more appropriate. A comparison of various methods is presented elsewhere ***(1, 2)***.

Perchloric acid (PCA) Extraction

Perchloric acid extraction extract water-soluble metabolites from the cells for analysis using NMR spectroscopy.

1. Resuspend the cell pellets in ~1 mL of 6% (v/v) PCA. Cells, unlike tissues, do not have to be homogenized. However, for maximum recovery, a homogenization setup (e.g. FASTPREP, MP Biomedicals) may be employed.
2. After the resuspending the cells completely in PCA (and homogenized, if preferred), centrifuge the tubes at 4°C for 30 min at 10,000 g. Transfer the supernatant to a new tube and discard the pellet (mostly cell debris).
3. Using 15M KOH, adjust pH of the solution to 2 – 3. If pH of the solution pH is greater than 8, use PCA to neutralize.
4. Once pH range of 2 – 3 is reached, switch to using 1M (or 0.1 M) KOH. Continue adding base until the pH is in the range of 6.5 – 7.5.
5. Centrifuge the tubes at 4°C for 30 min at 10,000xg. Transfer the supernatant to a new tube and discard the pellet. At this point, the pellet should be predominantly potassium perchlorate.
6. Lyophilize the samples to dryness.
7. Dissolve the lyophilized powder in approximately $1/4^{th}$ the original volume of ultrapure water. For example, if 1 mL of 6% (v/v) PCA was added to re-suspend the cell pellet, dissolve the lyophilzed powder in 250 µL in this step.
8. In most cases, pH of the solution will be between 8 – 8.5.
9. Use 0.1M NaOH and 0.1M HCl to adjust the pH of the solution to 7. Using 20 µL micropipettes to adjust pH is optimal for this step.
10. Centrifuge the sample for 15 min at 4°C at 10,000xg. Transfer the supernatant to a new tube and discard the pellet.
11. Lyophilize the sample to dryness.

For extracting fat-soluble metabolites (e.g. lipids), Folch's (methanol-chloroform) extraction may be utilized ***(3)***.

Steps 7 – 11 are for reducing inorganic salt content from the final sample. In cases where high throughput is paramount, these steps can be ignored in favor of achieving a tighter final pH (e.g. 6.95 – 7.05) in step 4.

It is beneficial to adjust pH on ice since lower temperature promotes more efficient desalting due to lower solubility of salts.

Refs

1. Lin CY, Wu H, Tjeerdema RS, et al (2007) Evaluation of metabolite extraction strategies from tissue samples using NMR metabolomics. Metabolomics 3:55–67

2. Martineau E, Tea I, Loaëc G, et al (2011) Strategy for choosing extraction procedures for NMR-based metabolomic analysis of mammalian cells. Anal Bioanal Chem 401:2133

3. Folch J, Lees M, and Stanley GHS (1957) A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. J Biol Chem 226:497–509